Effects of Environmental Tobacco Smoke on Susceptibility of Mice to Respiratory Infections

Dr. Trenton R. Schoeb

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CENTER FOR INDOOR AIR RESEARCH
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APPLICATION FOR RESEARCH CONTRACT

1. PRINCIPAL INVESTIGATOR. NAME, TITLE, TELEPHONE # AND MAILING ADDRESS. REDACTED

(A) Trenton R. Schoeb — NAME
(B) Associate Professor — TITLE
(C) — TELEPHONE #/ FAX #

(D) Comparative Medicine — DEPARTMENT
(E) University of Alabama at Birmingham — INSTITUTION

REDACTED REDACTED

2. PROJECT TITLE. Effects of Environmental Tobacco Smoke on Susceptibility of Mice to Respiratory Infections.

3. KEY WORDS. PLEASE PROVIDE THREE (3) KEY WORDS WHICH WILL BE USED AS REFERENCE HEADINGS.
Environmental Tobacco Smoke, Mice, Respiratory Infections

4. INSTITUTION. NAME AND ADDRESS OF INSTITUTION RESPONSIBLE AND ACCOUNTABLE FOR DISPOSITION OF FUNDS AWARDED ON THE BASIS OF THIS APPLICATION.

(A) University of Alabama at Birmingham — INSTITUTION
(B) UAB Station — STREET ADDRESS
(C) Birmingham — CITY
(D) AL 35294-2010 — STATE/ZIP

5. LOCATION. LIST LOCATION WHERE RESEARCH WILL BE CONDUCTED *IF OTHER THAN INSTITUTION IDENTIFIED IN #4 ABOVE.*

(A)

(B)

6. INCLUSIVE DATES AND TOTAL COSTS OF THIS SPECIFIC PROJECT RELATED TO EACH 12 MONTH PERIOD IF MORE THAN ONE YEAR IS REQUIRED TO COMPLETE PROJECT. SUMMARIZE FROM BUDGET PAGE, ITEM 12(J). IT MUST BE UNDERSTOOD THAT AWARDS FOR 2ND AND 3RD PERIODS ARE DEPENDENT ON CENTER APPROVAL OF CONTINUATION APPLICATION.

	Inclusive Date			Total Cost
(A) 1ST 12 MONTH PERIOD	1/1/93	THRU	12/31/93	$ -
(B) 2ND 12 MONTH PERIOD IF REQUIRED	1/1/94	THRU	12/31/94	$ REDACTED
(C) 3RD 12 MONTH PERIOD IF REQUIRED	1/1/95	THRU	12/31/95	$ -

7. INSTITUTIONAL OFFICER. NAME, TITLE AND TELEPHONE NUMBER OF INDIVIDUAL AUTHORIZED TO SIGN FOR THE INSTITUTION IDENTIFIED IN #4 ABOVE. IT IS UNDERSTOOD THAT THE OFFICER, IN APPLYING FOR A CONTRACT, HAS READ AND FOUND ACCEPTABLE THE CENTER'S MANAGEMENT OF RESEARCH CONTRACTS AND CONTRACT ADMINISTRATION POLICY.

(A) Kenneth M. Pruitt, PhD — NAME
(B) Associate VP for Research — TITLE
(C) — TELEPHONE REDACTED
(D) Kenneth M Pruitt — SIGNATURE OF INSTITUTIONAL OFFICER
(E) 5/29/92 — DATE

8. PRELIMINARY STUDIES*.
(A) FEASIBILITY OF PROPOSED RESEARCH
(B) QUALIFICATIONS OF INVESTIGATOR

9. EXPERIMENTAL PLAN*.
(A) DESIGN
(B) METHODS
(C) ANALYSIS OF DATA
(D) INTERPRETATION OF RESULTS
(E) TIMETABLE FOR THE INVESTIGATION
(F) LITERATURE CITED

CONFIDENTIAL
FOR REVIEW ONLY

10. AVAILABLE FACILITIES AND RESOURCES.

11. OTHER SUPPORT

* APPEND AS MUCH MATERIAL AS REQUIRED. TYPE, SINGLE SPACE. USE 8-1/2" X 11" WHITE PAPER AND LABEL EACH SHEET WITH NAME OF THE PRINCIPAL INVESTIGATOR IN THE UPPER RIGHT HAND CORNER AND PAGE NUMBER AT THE BOTTOM. CONSECUTIVELY NUMBER EACH ADDENDUM BEGINNING WITH PAGE 5. DO NOT INSERT PAGES BETWEEN PAGES 1 AND 6, E.G., 2A, 2B, 3A, ETC. INCLUDE NINE COPIES AND AN ORIGINAL. IF SENDING PHOTOGRAPHS, INCLUDE 2 ORIGINAL SETS. NOTE: EACH OF THE NINE COPIES MUST BE PLACED IN A BINDER PER MAILING INSTRUCTIONS.

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THE UNIVERSITY OF ALABAMA AT BIRMINGHAM
Office of the Vice President for Health Affairs
Institutional Animal Care and Use Committee

PENDING NOTICE

DATE: 05/28/92

INVESTIGATOR: SCHOEB, TRENTON R.

TITLE: EFFECTS OF ENVIRONMENTAL TOBACCO SMOKE ON SUSCEPTIBILITY OF MICE TO RESPIRATORY INFECTIONS

AGENCY: CENTER FOR INDOOR AIR RESEARCH

The above project is pending review before the Institutional Animal Care and Use Committee (IACUC). Review will occur on or before the next regularly scheduled monthly meeting of the IACUC. The results of the Committee's decision will be furnished to the investigator upon completion of the review. UAB is a fully accredited AAALAC institution. Please forward this notice to the appropriate granting agency.

Dee Nabors

Dee Nabors
IACUC Administrator

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The University of Alabama at Birmingham
BIC Volker Hall • 1717 Seventh Avenue South • UAB Station
Birmingham, Alabama 35294-0019 • (205) 934-3553 • FAX (205) 934-1188

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12. BUDGET. Detailed specific needs for the first 12-month period. Estimate category sub-totals for 2nd and 3rd periods, if required. Append justifications.

(a) Salaries. List personnel by name and title. Indicate individuals' % time to be spent on this project.	1st period	2nd period	3rd period
% Professional:			
15 Trenton R. Schoeb	$		
5 Jerry K. Davis			
5 Maureen K. Davidson		REDACTED	REDACTED
5 H. Kenneth Dillon			
% Technical:			
100 Kathryn C. Kervin	$		
100 Linda C. Thomas			
% Other:			
Fringe benefits payable at institution's rate of 26%			
Category (a) Sub-Total	$	REDACTED	REDACTED
(b) Consultants (per diem, travel & expenses):			
Joe McCain, PhD @ $117/hr	$		
Category (b) Sub-Total	$	REDACTED	REDACTED
(c) Supplies & Expense:			
Consumables (by category)			
smoking supplies	$ 4,000.00	$ 4,240.00	$ 4,494.40
Media, plasticware, etc.	3,500.00	3,710.00	3,932.60
Animals and related costs			
breeders	4,015.00	4,015.00	4,015.00
experimental	6,967.00	6,967.00	6,967.00
isolator supplies	2,000.00	2,120.00	2,247.20
Other expenses (itemize) gasses & standards, histology, serology, GC-MS supl.	5,110.00	6,826.60	6,056.20
office	800.00	848.00	898.88
Category (c) Sub-Total	$ 26,392.00	$ 28,726.60	$ 28,611.28
(d) Travel Expenses:			
	$	REDACTED	REDACTED
Category (d) Sub-Total			
(e) Alterations and Renovations:			
none			
Category (e) Sub-Total			
(f) Sub-contracts			
none			
Category (f) Sub-Total			
(g) Equipment			
See budget justification for itemization			
Category (g) Sub-Total			
(h) TOTAL DIRECT COSTS		REDACTED	REDACTED
(i) Indirect costs not to exceed 25% of the sum of (a) thru (f):			
(j) TOTAL PROJECT COSTS			

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13. BIOGRAPHICAL SKETCH of all professional personnel listed in 12(a). Append. Please include the following: Name, title, education, scientific field, major research interest, research and/or professional experience and publications. (Limit list of publications to the 20 most important and/or relevant.)

14. a) Are HUMAN SUBJECTS to be used in this research? _____ Yes ___X___ No
If yes, attach Institutional Review Board approval for procedures involving human subjects.

b) Are LABORATORY ANIMALS to be used in this research? ___X___ Yes _____ No
If yes, attach Institutional Animal Care and Use Committee approval for procedures involving animals.
Approval is pending; documentation will be forwarded when approval is received.

15. SIGNATURE OF PRINCIPAL INVESTIGATOR: It is understood that the applicant in applying for a Contract has read and found acceptable the Statements of Policy and Terms Under Which Project Contracts Are Made appearing in the application package.

Trenton R Schoeb _____ 5/20/92
Signature of Principal Investigator Date

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RESEARCH ABSTRACT

Title of Project: Effects of Environmental Tobacco Smoke on Susceptibility of Mice to Respiratory Infections

Investigator(s): Trenton R. Schoeb, DVM, PhD (PI); Jerry K. Davis, DVM, PhD; Maureen K. Davidson, PhD

Institution: University of Alabama at Birmingham
University Station
Birmingham, AL 35294

ABSTRACT: In the space below, please provide a descriptive summary of your proposed research project.

Results of epidemiologic studies suggest that exposure to environmental tobacco smoke (ETS) can increase the incidence and severity of infectious respiratory diseases, especially in children. However, these results are not consistent from study to study and are subject to a variety of potential confounding variables. In studies now in progress under a current CIAR contract, we have established methods for exposing mice to simulated ETS and assessing effects of ETS exposure on expression of Sendai virus and *Mycoplasma pulmonis* disease in young adult mice by quantifying numbers of the respective agents and by analysis of lesions by scoring and computerized morphometry. Results to date indicate that chronic, pre-infection exposure of young adult mice to ETS results in increased numbers of *M. pulmonis* organisms in the lungs within 7 days after inoculation. Experiments to meet one objective of the proposed work will be a direct continuation of the current project. Young adult mice will be exposed to ETS for 8 weeks before aerosol inoculation with *M. pulmonis* and studied for intervals of up to 3 months after inoculation to determine whether exacerbation of acute disease leads to increased severity of chronic lung disease and whether continued ETS exposure potentiates any such effect. We also use will an established method of aerosol inoculation with radiolabelled *M. pulmonis* to assess effects of chronic ETS exposure on intrapulmonary killing and physical removal of *M. pulmonis* from the lungs, as these are the most likely mechanisms by which ETS exposure could lead to increased numbers of organisms in the lungs at short intervals after infection. Additional questions concerning association of tobacco smoke exposure with increased respiratory disease in children are whether such associations are due to in utero effects of maternal smoking or ETS exposure or to postnatal exposure of the child to ETS. Similar methods will be used to meet the remaining objectives of the proposed work, which will be to assess effects of ETS exposure of female mice before and during gestation on susceptibility of their offspring to *M. pulmonis* and Sendai virus; to determine whether exposure of neonatal mice to ETS increases susceptibility of suckling and adolescent mice; and to test whether prenatal and postnatal exposure act synergistically.

Trenton R Schoeb — 5/28/92

Signature, Principal Investigator — Date

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8a. AIMS

The risk of developing respiratory infections, asthma, or decreased pulmonary function appears to be increased in children of parents who smoke (1-7). However, it is unclear whether this is due to postnatal exposure to environmental tobacco smoke (ETS), *in utero* effects of maternal smoking or ETS exposure, or a combination of these (6,8-11).

During the last two years, we have established a model for exposure of mice to simulated ETS, and have examined the effects of simulated ETS on susceptibility to acute infection, using both murine respiratory mycoplasmosis (MRM) and Sendai virus infection in young adult mice. Results to date indicate that ETS exposure increases the numbers of *Mycoplasma pulmonis* organisms in the lungs within seven days after inoculation with low doses of mycoplasmas.

(A) Hypotheses

We will test the hypotheses (i) that differences due to ETS exposure in expression of acute, low-dose mycoplasmal disease lead to increased severity of chronic lung disease; (ii) that increased numbers of organisms in the lungs of mice exposed to ETS are due to alterations in pulmonary clearance, and (iii) that ETS exposure during gestation or early postnatal life results in greater differences in susceptibility to *M. pulmonis* or Sendai virus disease than does exposure of adult mice.

(B) Objectives

Our objectives will be (i) to determine the effects of chronic ETS exposure on low-dose mycoplasmal disease up to 3 months after infection; (ii) to determine whether the differences due to ETS exposure in numbers of *M. pulmonis* organisms seven days after inoculation with low doses of organisms is due to changes in pulmonary clearance mechanisms; (iii) to determine whether exposure of pregnant mice to simulated ETS increases susceptibility of their offspring to *M. pulmonis* or Sendai virus; and (iv) to determine whether exposure of neonatal mice to ETS increases susceptibility to *M. pulmonis* or Sendai virus, and (v) to determine whether prenatal and neonatal exposure to ETS act synergistically to increase susceptibility to acute respiratory infections. Accomplishing the first two objectives will allow us to more completely characterize effects of ETS in young adult mice, whereas the last three objectives will allow us to assess effects of prenatal or early postnatal ETS exposure on susceptibility to respiratory infections.

8b. SIGNIFICANCE OF PROPOSED WORK

(A) Background & literature

Environmental Tobacco Smoke (ETS). ETS is the term used in the 1986 reports of

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the Surgeon General (12) and the National Research Council (13) to characterize the tobacco combustion substances inhaled by nonsmokers. ETS consists of sidestream smoke, which is emitted from the burning tip of the cigarette, and mainstream smoke, which is inhaled, filtered, and exhaled by the smoker. ETS is composed mostly of sidestream smoke, the composition of which is different from that of mainstream smoke because of the higher temperature of combustion at formation and because sidestream smoke is unfiltered. Sidestream smoke is qualitatively more hazardous than mainstream smoke (6), and 10 to 20 percent of the particles in sidestream smoke could be deposited in respiratory airways (14).

Relationship of ETS to respiratory disease in children. Epidemiologic studies consistently have shown associations between exposure to ETS and increased incidence of respiratory infections in young children (3-5,7,11-31). Increased risk in children is particularly worrisome because epidemiologic evidence also has indicated that childhood respiratory illness is an important risk factor for chronic obstructive pulmonary disease in later life (32-34). The Surgeon General concluded that children of parents who smoke have more respiratory infections, more respiratory symptoms, increased frequency of hospitalization for bronchitis and pneumonia, and a smaller rate of increase in lung function than children of parents who do not smoke (12). Furthermore, the National Research Council's report noted that nearly all relevant cross-sectional studies found increased prevalence of respiratory symptoms among children of smoking parents compared with those of nonsmoking parents (13).

However, in many of these studies, various confounding factors were not considered, such as socioeconomic status, use of gas stoves, parental symptoms of cough, the child's birth weight, number of siblings, and, in older children, the child's own smoking (22). This may have led to bias in favor of finding associations, even if no true causal relationships existed. Even if causal relationships do exist, they may not be straightforward. For example, increased incidence of respiratory infections consistently shows a stronger association with maternal smoking than with paternal smoking. In a national study of 12,743 children in England, maternal, but not paternal, smoking had a significant influence on the reported incidence of bronchitis and admission to hospital for lower respiratory tract illness during the first five years of life (11). However, reported rates of admissions were found to be as high in children of mothers who stopped smoking during pregnancy as in those whose mothers smoked continuously both during and after pregnancy. Furthermore, rates of hospital admission for lower respiratory tract diseases in children whose mothers started smoking only postnatally were no higher than in those whose mothers remained nonsmokers. These findings suggest that the association between maternal smoking and respiratory disease is real, but that it is mediated mainly through a congenital effect, not passive exposure after birth. Other investigators also have suggested that the effects of maternal smoking on respiratory disease are related to congenital effects (1,10,22).

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Effects of congenital exposure to ETS. The adverse effects of maternal smoking during pregnancy on the fetus are well established. Offspring of smoking mothers tend to be smaller in size, and have an increased risk for low birth weight, perinatal mortality, and congenital malformations (1,2,9,35-38). No definitive information is available concerning effects of ETS exposure on fetal development and on long-term consequences, but there is suggestive evidence that ETS might also affect the fetus. Increased amounts of cotinine, a metabolic derivative of nicotine which is widely accepted as a short-term marker for ETS exposure in nonsmokers (39), have been detected in the amniotic fluid of wives of smokers (40). Furthermore, serum cotinine concentrations of between 1.0 and 9.9 ng/ml in the serum of pregnant nonsmokers during the second trimester have been related to a lower mean birth weight (9). However, in only three of eight studies were there significant decreases in birth weights of infants born to nonsmoking mothers and smoking fathers (9). More consistent effects were found in studies which also considered the length of time the mother was exposed to ETS (9). Still, the relative risk for low birth weight for nonsmoking women is generally 1.5 or less. There also are studies that suggest that exposure of mothers to ETS may increase perinatal mortality, induce malformations, and increase the cancer rate in offspring. In fact, it has been hypothesized that passively inhaled smoke may be more dangerous for the fetus than active smoking on a per dose basis (1). Maternal smoking induces placental enzymes that may partially protect against the teratogenic and carcinogenic effects of compounds in tobacco smoke (1,9). These enzymes are induced to a much lower extent by passively inhaled tobacco smoke, which may allow a higher percentage of compounds to cross the placenta. Furthermore, due to incomplete combustion, sidestream smoke contains a higher concentration of toxic compounds than does mainstream smoke (6). Clearly, compounds found in ETS can reach the fetus. These may affect development of the respiratory tract, as suggested by preliminary studies showing a decrease in pulmonary function during the first three days of life in children born to mothers exposed to passive smoke (41). Also, effects on respiratory defense mechanisms could occur even if overt loss of pulmonary function was not evident.

Effects of ETS on immune mechanisms. Little information is available on the effects of ETS on immunity, but subchronic and chronic exposure of animals to mainstream smoke leads to changes indicative of immunosuppression. Lymphocyte proliferation in response to the mitogens PHA and LPS is decreased, suggesting compromise of cell function (42). Antibody production can be suppressed (42). Furthermore, animals chronically exposed to mainstream smoke have a higher incidence of infectious diseases after challenge with viruses. Localized immunological changes in the lung can include reduction of bronchus-associated lymphoid tissue and immunoglobulin concentrations, changes in pulmonary macrophage function, and alterations in mucociliary function (42-57). In humans, smoking induces elevated leukocyte counts, increased cytotoxic/suppressor and decreased inducer/helper T-cell numbers, slightly suppressed T-lymphocyte activity, significantly decreased natural killer cell activity, lowered circulating immunoglobulin titers, (except for IgE, which is elevated), and increased susceptibility to

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infection (42). A few studies have indicated a potential for ETS to induce hypersensitivity and suppress immunoregulatory substances (42,58). In contrast, other investigators have failed to detect immunological changes associated with environmental smoke (42). In summary, there is ample evidence that tobacco smoke can alter both local and systemic immune mechanisms; ETS may have similar effects, especially in neonates or premature infants, whose pulmonary defense mechanisms are less efficient than those of adults (59-69).

(B) Identification of gaps in proposed research area

Current evidence linking ETS to increased incidence or severity of respiratory disease in children is primarily circumstantial. Epidemiologic studies show that there is an association between maternal smoking and increased incidence of respiratory disease in children; they do not prove cause and effect. The possibility that the association is due to a congenital effect remains a hypothesis; there is no evidence. In contrast, epidemiologic data and results of animal studies show convincingly that NO_2 exposure can increase the incidence and severity of respiratory disease. As with NO_2 and other oxidant gases, animal studies will be necessary to determine whether prenatal or neonatal exposure to ETS adversely affects respiratory defense mechanisms.

Mechanisms of respiratory tract defense are complex, and both nonspecific and immune mechanisms almost certainly are required for maximum resistance. Furthermore, there is little reason to assume that the same mechanisms operate to the same degree in all stages of disease; those responsible for preventing infection probably are not the same as those required for recovery. For example, concentration on alveolar macrophages and the mucociliary apparatus as the major nonspecific defenses of the respiratory tract may have resulted in other, possibly equally important, defensive mechanisms being overlooked. It should also be pointed out that defense mechanisms in the upper respiratory tract are poorly understood, may be different from those of the lung, and may respond differently to pollutants. Second, it is almost certain that defenses against different organisms require different combinations of mechanisms (70). Thus, results of studies with one organism cannot be assumed to apply to all others. Furthermore, studies of defenses against a human pathogen in an animal species that normally is not susceptible to the organism may provide misleading results, because the primary resistance mechanism or mechanisms in the animal host may be different from, or more efficient than, the human counterparts. Third, most effects of pollutants on respiratory defense mechanisms have not been shown to be responsible for increased incidence or severity of respiratory disease in animal models. Furthermore, ETS and inhaled mainstream smoke are quantitatively and qualitatively different. Therefore, it is uncertain whether results from studies comparing smokers and nonsmokers, or of animals exposed to mainstream smoke, are applicable to ETS.

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(C) Project importance

Experimentally induced infections with *M. pulmonis* and Sendai virus provide excellent animal models to study infectious respiratory disease, and offer several advantages which offset the theoretical disadvantage that they are not exact analogues of specific human diseases (71-77). Lesions, which occur in the upper and lower respiratory tract, can be modified by various intrinsic and extrinsic factors (71-74,76-88). Methods for quantifying susceptibility to MRM and Sendai virus by both dose-related parameters and by morphometric analysis of lesions have been developed (76,80-83,89), and extensive data is available on local and systemic host defenses operative against both *M. pulmonis* and Sendai virus (71-73,90-112). Finally, each disease bears close similarities to certain common human respiratory diseases. Lesions of experimental Sendai virus infection in mice resemble those of influenza (90,107,113), and *M. pulmonis* infection resembles *Mycoplasma pneumoniae* infection in the obligate association of the causative organism with the respiratory epithelium and the general characteristics of the inflammatory response (74). Therefore, use of MRM and Sendai virus infection in mice will allow us to examine a wide range of potential targets by which ETS could affect disease severity in both the upper and lower respiratory tract.

To our knowledge, the studies supported by Dr. Davis's current CIAR contract provide the first experimental evidence of an effect of ETS exposure on expression of any respiratory disease. The proposed studies will directly extend these observations and determine whether increased numbers of organisms in the lung can be directly linked to increased severity of pulmonary disease. They also will examine the most likely mechanisms by which ETS exposure could lead to increased susceptibility. In addition, the experiments proposed for specific aims (iii), (iv), and (v) will provide the first experimental study of the relative importance of prenatal and postnatal exposure to ETS on susceptibility to respiratory infectious disease. Inasmuch as general mechanisms of pulmonary defenses appear to be similar in man and animals, identification of effects of ETS on pulmonary defense mechanisms in these animal models should indicate host defense mechanisms that are likely targets of ETS exposure in man. These mechanisms can then be directly examined in epidemiologic studies to test whether or not ETS is associated with adverse effects on respiratory defenses, and thus with increased risk of respiratory infections in humans. This approach would bypass the need for extrapolation on a per-dose basis from rodents to humans.

8c. PRELIMINARY STUDIES

During the last two years, we have established a model for exposure of mice to simulated ETS. We have examined the effects of simulated ETS on susceptibility to acute infection, using both MRM and Sendai virus infection in young adult mice. Also during this time, we have developed and standardized the methods for aerosol infection of mice with

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Sendai virus.

(A) Exposure of adult mice to simulated ETS

We have modified the nose-only exposure system developed at the Tobacco and Health Research Unit of the University of Kentucky (114,115) to deliver aged sidestream smoke. We use 1R4F reference cigarettes, which approximate modern filtered cigarettes. Cigarettes are smoked at the rate of one puff per minute and 12 puffs per cigarette. Air flow through the exposure system is 2 l/min. Modifications made to improve characterization and quality control of smoke exposures include addition of a hood for collection of effluent smoke for analysis of particle sizes with an electronic aerosol size analyzer (EASA) and a MOUDI cascade impactor, provision for data reduction and analysis of particle size data, and provisions for testing CO and nicotine content of the effluent smoke. A custom made diluter that uses a system of baffles to mix sidestream smoke and compressed air was added, allowing smoke dilutions of 1:1 to 1:500. A humidification chamber is used to standardize the relative humidity of the smoke delivered to the exposure chamber. The humidifier water is monitored by repeated culture for bacterial growth.

(B) Characterization of simulated ETS exposure

Naturally occurring ETS varies widely in concentration and composition; however, the concentration of smoke that induces approximately 10% carboxyhemoglobin (COHb) in mice appears to be similar to that found in smoky bars and other places where ETS is at the highest concentration (39).

Carboxyhemoglobin (COHb). Animal exposures are based on COHb concentrations in the mice, with target concentrations of 1%, 5%, and 10% COHb as determined by spectrophotometry (115). COHb concentrations reach a plateau after mice have been exposed to simulated ETS for 100, then remain stable for the remainder of a 4 hour exposure.

Particulate size distribution. Continual monitoring with the EASA is used to determine when smoke reaches the animal ports and has reached "steady state." Approximately 20 minutes (which also represents the average aging time of the sidestream smoke) are required for the smoke in the exposure chamber to reach equilibrium, at which time animals are placed in the animal ports. At least in the size range to which the EASA is sensitive, there does not seem to be any major change in particle size distribution regardless of the dilution of smoke. All three dilutions of smoke have also been evaluated using the MOUDI impactor, and the distribution of particle sizes is about the same for all dilutions with this method. The curves are tri-modal for all three dilutions, and most of the particles are in the respirable size range (greater than 90% of the particles are less than 2 μm in size). The mass median aerodynamic diameter (MMAD)

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values are: 1:18 dilution, MMAD = 0.518, σ_g = 2.35; 1:70 dilution, MMAD = 0.452; σ_g = 2.049; and 1:335 dilution, MMAD = 0.713, σ_g = 2.21.

Carbon monoxide (CO) concentration. CO concentrations in the exposure chamber have been determined for various dilution of smoke. At a 1:18 dilution, CO concentrations are 13 ± 4 ppm; at 1:70, they are 7 ± 4 ppm; and at 1:335 they are 2 ± 0.3 ppm. There is no difference in the CO concentration among the 4 chimneys on the smoking machine, regardless of whether or not mice are in the exposure chamber.

Particulate matter. Cambridge filters inserted downstream from the animal ports are used to measure total particulates. Since ETS has several volatile components, weights are calculated immediately after each run. At the lowest dilution of smoke (that corresponding to 10% COHb in the mice), the concentration of particulates in simulated ETS is approximately 1300 $\mu g/m^3$ without animals and 750 $\mu g/m^3$ with animals.

Nicotine and other organic compounds. Nicotine is collected using XAD-4 sorbent tubes and measured at Southern Research Institute according to an established gas chromatographic method (116). At the concentration of smoke inducing 10% COHb, nicotine concentrations without mice in the exposure apparatus average 32 $\mu g/m^3$ and 30 $\mu g/m^3$ when mice are present. Selected other organic compounds, including ethyl benzene, 1,4-dimethylbenzene, 1,3,5,7-cyclooctatraene, 1,3-dimethylbenzene, trimethylbenzene, D-limonene, decamethylcyclopentasiloxane, dodecamethylcylohexasiloxane, and trisiloxane, also are monitored to ensure consistency of smoke composition.

(C) Effects of ETS on Sendai virus infection in adult mice

Ninety-six, pathogen-free, 8-10 week old, C57BL/6N mice of both sexes were used to determine the effects of chronic ETS exposure on acute Sendai virus infection. Half of the mice were exposed for 3 hrs per day for 5 days/week for 8 weeks to the ETS concentration that induced 10% COHb. Control mice were sham exposed. The average mass median volume diameter (MMVD) was 0.13 μm (σg = 2.1), and the MMAD was 0.6 μm (σg = 2.4). The average concentration of CO in the effluent smoke (measured without animals) was 9 ± 3 ppm and the relative humidity was 68 ± 7%. COHb concentrations in ETS exposed mice averaged 9 ± 2%, average COHb for control mice was 1 ± 1%. Nicotine concentrations averaged 28 $\mu g/m_3$, and total particulates averaged 7.6 ± 3.8 mg/m_3. After 8 weeks of ETS exposure, 24 mice from each group were exposed to 2 ppm NO_2 for 4 hours; the remaining mice in each group were sham exposed. All mice were exposed to aerosols of Sendai virus generated from undiluted stock ($TCID_{50}$ = 10^4/ml).

Twelve mice from each group were killed at 3 days post inoculation (PI) and the remaining mice were killed at 5 days PI. On each day, 6 mice were processed for virus titers in the upper and lower respiratory tract. Tissues were taken from the remaining 6

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mice for histology. Serum was taken from all mice for virus and mycoplasma serology. There were no differences in disease expression among the groups.

(D) Effects of ETS on MRM

The effects of ETS on MRM were determined in experiments of similar design, except that animals were exposed to aerosols generated from different concentrations of viable *M. pulmonis* in the nebulizer and the mice were killed 7 days PI. In two separate experiments, the characteristics of the simulated ETS were nearly identical with those above. Initially, mice were exposed to *M. pulmonis* doses approximating the 50% infectious dose, i.e, 5×10^5 and 5×10^4 CFU/ml in the nebulizer. At these doses, there were no differences in lesions or numbers of *M. pulmonis* among the different groups. When animals were exposed to doses approximating the 50% gross pneumonia dose (PD_{50}), i.e., 10^{10} CFU/ml and 10^9 CFU/ml in the nebulizer, there were no differences among groups infected with the higher dose. However, both ETS and NO_2 altered the distribution of organisms in mice exposed to aerosols generated with 10^9 CFU/ml in the nebulizer. At this dose, *M. pulmonis* was found in the lungs only in mice exposed to ETS. Mice exposed to 2 ppm NO_2 had fewer *M. pulmonis* in the nasal passages (Appendix, Fig 1). An increase in the number of bacteria that can be cultured from the lungs of smokers has been shown previously and is thought to be related to the effects of tobacco smoke on ciliary motility (117). The increase in *M. pulmonis* in the lungs of mice following low dose infection could be due to the effects of simulated ETS on ciliary motility, but further experimentation will be required.

Studies are underway to determine whether concentrations of simulated ETS inducing 5% or 1% COHb also increase the number of *M. pulmonis* organisms in the lungs after low dose infection. In addition, studies will be completed under Dr. Davis's current contract to determine whether ETS exacerbates lesions in mice with chronic MRM.

(E) Pollutant effects on pulmonary clearance

In previous studies, we have examined the effects of NO_2 on MRM (Appendix, Manuscripts 1 and 2). While NO_2 does not appear to be synergistic with ETS and will not be used in the proposed studies, similar methods will be necessary to characterize the effects of ETS. In brief, we have found that exposure of C57BL/6N mice to 5 ppm or more of NO_2 for 4 hours increased mortality, severity of lung lesions, and numbers of *M. pulmonis* in the lungs. These effects appeared to be mediated by decreased intrapulmonary killing, probably due to effects on pulmonary macrophages. Although it is unlikely that ETS at the concentrations employed also affects alveolar macrophages, inasmuch as effects of ETS and NO_2 are not additive, studies similar to those described in manuscripts 1 and 2 (Appendix) will be required to determine whether the changes in mycoplasmal numbers in the lungs are related to changes in pulmonary clearance.

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Other studies relevant to this application concern differences between mouse strains in susceptibility to *M. pulmonis*, development of an aerosol model for infecting mice with *M. pulmonis*, and studies of immune responses to *M. pulmonis* in both the upper and lower respiratory tract (Appendix, Manuscripts 3-6). These provide evidence of expertise in the techniques that either will be employed in the proposed work or, if effects of ETS exposure on susceptibility are found, would be required to determine the defense mechanisms affected by ETS.

11. EXPERIMENTAL PLAN

(A) Design

Determination of effects of chronic ETS exposure on low-dose mycoplasmal disease [specific aim (i)]. Studies in progress under Dr. Davis's current contract were designed to address (i) effects of chronic, *pre-infection* ETS exposure on *acute* disease, i.e., effects on susceptibility to establishment of infection and progression of early stages of an infectious disease; and (ii) effects of chronic, *post-infection* ETS exposure on *chronic* disease, i.e., effects on progression of a pre-existing infection. Results to date have shown that exposure to ETS for 8 weeks before inoculation results in increased numbers of *M. pulmonis* organisms in the lungs at 7 days PI. Previous studies in our laboratory have shown that severity of chronic MRM can be profoundly influenced by factors acting in the early stages of the disease, such as acute viral infections, exposure to NO_2, and differences in *M. pulmonis* dose (77-79,120; Manuscripts 1 & 2, Appendix). Therefore, differences in expression of acute MRM could lead to large differences in severity of the chronic disease, especially if ETS exposure is continued. The experiments proposed in this application will determine whether chronic exposure to ETS prior to infection affects development of chronic disease, especially if coupled with continued exposure after inoculation. This experimental exposure should closely mimic that experienced by children exposed to ETS, in that exposure probably occurs both prior to infection and throughout the course of the disease.

Forty-eight C57BL/6N mice will be exposed 3 hours per day, 5 days per week, to the concentration of simulated ETS that induces 10% COHb. Forty-eight mice will be sham exposed. After exposure to ETS for 8 weeks, both groups of mice will be exposed by aerosol to *M. pulmonis* at the 0.1 PD_{50} dose. Twelve mice will be killed at 2, 4, 8, and 12 weeks PI. The respiratory organs of 6 mice in each group will be prepared for histologic examination and those of the other 6 will be quantitatively cultured for *M. pulmonis*. Lesions will be evaluated as described previously (Appendix, Manuscripts 3, 7-9). If exposure to this concentration of ETS increases respiratory lesions or numbers of organisms in the respiratory organs, the experiment will be repeated using the concentrations of ETS that induce 5% and 1% COHb. If subjective analysis of lesions indicates changes in susceptibility due to ETS exposure, lesions will be further analyzed by computerized morphometry (Appendix, Manuscripts 7-9).

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Determination of effects of chronic ETS exposure on pulmonary clearance mechanisms [specific aim (ii)]. Studies of pulmonary clearance have been used extensively to characterize effects of pollutants on respiratory tract resistance to bacterial challenge (70,121,122). In such studies, radiolabelled bacteria are delivered to the lungs by aerosol. Radioactivity and numbers of viable organisms are determined immediately after exposure and at intervals thereafter, with loss of radiolabel indicating physical removal of the organisms (via either the mucociliary apparatus or lymphatic vessels), and decreases in the ratio of colony forming units to radioactive decompositions per minute (CFU/DPM) indicating intrapulmonary killing of the bacteria. We have used this model to show that decreased rates of intrapulmonary killing are associated with greater susceptibility of C3H/HeN mice to MRM than C57BL/6N mice, and with increased severity of MRM in C57BL/6N mice exposed to NO_2 (Appendix, Manuscripts 1, 2, & 10).

Studies of effects of ETS exposure on pulmonary clearance have not been reported; however, active smoking decreases ciliary activity and alters mucus characteristics in both the tracheobronchial tree and nasal passages (117,123-127). The responsible mechanisms have not been determined, but reduction in number of cilia and changes in mucus composition appear most likely. Smoking also induces changes in alveolar macrophages (44,48,49,128); thus, it is plausible that tobacco smoke could adversely affect intrapulmonary killing of bacteria. The experiments outlined below will determine whether chronic exposure to simulated ETS alters either physical transport or intrapulmonary killing of *M. pulmonis*, the most likely mechanisms by which chronic exposure to ETS could lead to increased numbers of *M. pulmonis* in the lungs.

Initially, 12 C57BL/6N mice will be exposed 3 hours per day, 5 days per week, for 8 weeks to the concentration of simulated ETS that induces 10% COHb. Twelve mice will be sham exposed. After ETS exposure, all mice will be exposed to aerosols of radiolabelled, viable *M. pulmonis* and nasal and pulmonary clearance will be measured as described previously (11; Manuscript 10, Appendix). If either physical removal or intrapulmonary killing is altered in ETS exposed mice, we will conduct additional experiments of the same design to study effects of exposure to lower concentrations of ETS. We also will assess the time course of ETS-induced changes in pulmonary clearance using the lowest concentration of ETS that affects either physical removal or intrapulmonary killing. Forty-eight mice will be exposed to ETS for 8 weeks, and 12 mice will be used to assay nasal and pulmonary clearance at 2, 4, 8, and 12 weeks after exposure to ETS.

Determine whether exposure of pregnant mice to simulated ETS increases susceptibility of their offspring to Mycoplasma pulmonis *or Sendai virus [specific aim (iii)].* Several epidemiologic studies link parental, particularly maternal, cigarette smoking to various effects on fetal and child development (2,9,35,37,129-131). Increased risk of low birth weight is most consistent among these, but increased occurrence of both acute respiratory illnesses and chronic respiratory symptoms in children also have been

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reported (4,10,58,129,132). Respiratory effects seem to be greatest early in life and cannot be separated from *in utero* exposure (1,8,129,133). Although neither responsible mechanisms nor direct causal relationships have been established, the most widely favored hypothesis is that reduction in birth weight, and perhaps other effects of maternal smoking during pregnancy are related to fetal hypoxia (9). Effects of *in utero* ETS exposure on respiratory function in children are unknown. However, preliminary evidence suggests that maternal passive smoking during pregnancy may be associated with decreased pulmonary function in neonates (41), and similar effects may occur in rats (134). Inasmuch as tobacco smoke can have a variety of effects on the immune system (42) and passive smoking during pregnancy can result in exposure of the fetus to substances in tobacco smoke (40,135), it is reasonable that ETS exposure during pregnancy could result in subtle alterations in host resistance that could be reflected in changes in postnatal susceptibility to respiratory disease. The mouse is likely to be an appropriate model for study of these effects as mice and humans both have hemochorial placentae (136). The following experiments will test the hypothesis that exposure of mouse dams to simulated ETS during pregnancy results in increased susceptibility to respiratory infection in the offspring.

Twenty-four female C57BL/6N mice will be exposed to the concentration of ETS inducing 10% COHb for 3 hours/day, 5 days/week, for 5 weeks. Twenty-four mice will be exposed to CO in a whole body exposure chamber, which we have used previously to expose mice to NO_2 (Appendix, Manuscript 1 & 2). The concentration of CO will be that which induces 10% COHb. Twenty-four female mice also will be sham exposed with room air. All mice will be housed in the absence of males to suppress estrus (137). Male mice will then be introduced into each group of female mice, which will cause most of the female mice to breed within four days (137). ETS, CO, and sham treatments will continue until all of the mice have given birth. Litter sizes and weights of neonatal pups will be recorded. Neonatal mice will be randomized among the dams within each treatment group, and litter sizes will be standardized at 6 pups per dam.

At least 24 pups from each group (ETS, CO, controls) will be infected with the 0.1 PD_{50} dose of *M. pulmonis* by intranasal inoculation either within 24 hours after birth or at 14 or 28 days of age. (Intranasal inoculation will be used for inoculation of young mice because our present nose-only exposure apparatus cannot be used for mice this small. Preliminary experiments will be performed to determine the intranasal PD_{50} dose for C57BL/6N mice of each age.) Mice will not be exposed to ETS after birth. Twelve mice of each group will be killed 7 days PI and the remainder will be killed at 28 days PI. The nasal passages, tracheas, and lungs of 6 pups in each group will be prepared for histologic examination as previously described (82), and those of the other 6 will be quantitatively cultured for *M. pulmonis* (120,138). Lesions will be evaluated by scoring and morphometric analysis, as described previously (Appendix, Manuscripts 7-9). Experiments of a similar design will be used to determine whether prenatal exposure to ETS alters susceptibility to Sendai virus. Mice will be studied at 3 and 5 days PI, inasmuch as Sendai

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virus does not cause chronic disease. Viral titers in tissues will be determined by hemadsorption as previously described (118).

If exposure of dams to this concentration of ETS increases respiratory lesions in their offspring or leads to changes in the numbers of organisms that are found in the respiratory organs after inoculation with either *M. pulmonis* or Sendai virus, the experiment will be repeated using the concentrations of ETS and CO that induce 5 and 1% COHb in the dams. These experiments will determine whether ETS exposure during pregnancy alters susceptibility of the offspring to respiratory infection and whether any observed effect is likely to be due to hypoxia.

Determine whether exposure of neonatal mice to ETS increases susceptibility to M. pulmonis *or Sendai virus [specific aim (iv)].* It is well recognized that neonates are more susceptible to infections than older children, and a variety of phenomena related to host defenses, including neutrophil chemotaxis, intracellular killing of bacteria, and complement activation, are impaired in neonates (63,65-69). Although T lymphocytes are present in normal numbers, their response to antigens is somewhat slower than that of adult cells, and they produce less B cell stimulatory cytokines. Maturation of B lymphocytes into antibody-producing plasma cells also is somewhat slower. Therefore, it is plausible that effects of ETS exposure on susceptibility to infection may be particularly pronounced in neonates, or that ETS exposure could delay maturation of respiratory tract defenses. The experiments outlined below will determine whether chronic ETS exposure beginning at birth affects susceptibility of mice to respiratory infection.

Forty-eight C57BL/6N neonatal mice will be exposed to the concentration of ETS necessary to induce 10% COHb in adult mice. (The neonatal exposure system is described below.) Forty-eight pups will be sham exposed. After 14 or 28 days of ETS exposure, 24 mice in each group will be infected with the 0.1 PD_{50} dose of *M. pulmonis*. Twelve mice of each group will be killed at 7 days PI and the remainder at 28 days PI. The respiratory organs of 6 pups in each group will be prepared for histologic examination, and those of the other 6 will be quantitatively cultured for *M. pulmonis*. Experiments of a similar design will be used to determine whether neonatal exposure to ETS alters susceptibility to Sendai virus. If exposure of neonatal mice to this concentration of ETS exacerbates either respiratory disease, the appropriate experiment will be repeated with the concentrations of ETS and CO that induce 5% and 1% COHb.

Determine whether prenatal and neonatal exposure to ETS act synergistically to increase susceptibility to acute respiratory infections [specific aim (v)]. There is wide acceptance that maternal smoking decreases birth weight (9,132,133), and suggestive evidence that maternal exposure to ETS has a similar effect (8,9,35). Low birth weight infants have additional immunologic defects, such as decreased immunoglobulin levels in serum and secretions, including respiratory secretions (62,139). Although the major effect of maternal smoking is decreased birth weight for gestational age, there is some

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evidence that maternal smoking, and perhaps maternal ETS exposure (9), is a factor in premature birth. Immune defenses in premature infants are even less efficient than in normal neonates (59,60,140). Thus, maternal exposure to ETS during pregnancy and neonatal exposure after birth may be synergistic. The experiments outlined below will determine whether prenatal and postnatal ETS exposure has synergistic effects on expression of *M. pulmonis* or Sendai virus disease in mice. These experiments are done separately from those described above because of the limitation on the number of mice that can be exposed to ETS at a time.

Twenty-four female C57BL/6N mice will be exposed to the lowest concentration of ETS that induced any effects on *M. pulmonis* or Sendai virus infection in the experiments outlined above. If no effects were seen, the concentration inducing 10% COHb will be used. All exposures will be 3 hours/day, 5 days/week. Twenty-four female mice will be sham exposed with room air. After exposure for 5 weeks, timed pregnancies will be induced as described above, and ETS and sham treatments will continue until all of the mice have given birth. The total time of exposure of the female mice will thus be approximately 8 weeks. Litter sizes between the ETS and sham exposed dams will be standardized at 6 pups per dam. Furthermore, pups will be swapped between litters so that each dam exposed to ETS has 3 pups exposed to ETS *in utero* and 3 control pups.

If results of previous studies show a clear effect of ETS on susceptibility to respiratory infection, the times after birth and times PI at which effects were evident will be used in the following experiment. If no effects were observed, we will examine inoculations at two different ages and two different times PI. Whole body exposure will be used to expose mouse pups (see below) to the same concentration of ETS. This will give four treatment groups: (i) controls, (ii) prenatal ETS, (iii) postnatal ETS, and (iv) both prenatal and postnatal ETS. For each group, 24 pups will be infected with the 0.1 PD_{50} dose of *M. pulmonis* by intranasal inoculation at 14 days of age, and the same number will be infected at 28 days of age. Twelve mice of each group will be killed at 7 days PI and the remainder will be killed at 28 days PI. The respiratory organs of 6 pups in each group will be prepared for histologic examination, and those of the other 6 will be quantitatively cultured for *M. pulmonis*. Experiments of a similar design will be used to determine whether prenatal and postnatal exposure to ETS synergistically affect Sendai virus infection. If a strong synergistic effect is seen with prenatal and postnatal exposure to ETS, an abbreviated form of the experiment will be repeated with lower concentrations of ETS.

(B) Methods

Animals. The mice to be used in these studies will be C57BL/6N mice from a gnotobiotic breeding colony maintained since 1984 at the University of Alabama at Birmingham. The mice are housed in Trexler-type plastic film isolators, bred by brother-sister matings, given autoclaved tap water and autoclaved commercial diet for axenic

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rodents. The breeder mice are monitored for contamination by biannual fecal cultures and by necropsy examination of retired breeders, including histologic examination of all major organs. Procedures at necropsy also include cultures for bacterial pathogens and for mycoplasmas, and commercial serologic tests for Sendai virus, pneumonia virus of mice, mouse hepatitis virus, minute virus of mice, GD VII virus, reovirus-3, lymphocytic choriomeningitis virus, mousepox (ectromelia), K virus, mouse polyoma virus, mouse adenovirus, and rotavirus (epizootic diarrhea of infant mice). No evidence of murine pathogens has been found since the colony was established. During experiments, the mice will be housed in filter-top micro-isolator cages and given autoclaved food, water, and hardwood-chip bedding. Cage changing and other manipulations will be done under a laminar flow hood.

Animal inoculations. M. pulmonis strain CT will be grown to late log phase in mycoplasma broth for each experiment (141; Manuscript 7, Appendix). Numbers of CFU/ml in the inoculum will be determined by serial dilution and plating and will be verified by culture of an aliquot for each experiment. Inoculation of the mice with *M. pulmonis* will be done by exposing them for 30 minutes in a nose-only exposure chamber to an aerosol of *M. pulmonis* stock generated with a DeVilbiss model 45 nebulizer as we have described previously (83). The size of the aerosol particles generated is monitored with a seven-stage Mercer impactor and a six-stage Andersen microbial sampler to assure that the particles are small enough to reach the lower respiratory tract and that excessive loss of *M. pulmonis* viability does not occur during aerosolization.

Sendai virus stock will be grown in BHK-21 cells from seed stock obtained from American Type Culture Collection. Inoculations by aerosol will be done according to the protocol developed under Dr. Davis's current contract.

ETS exposure. Adult mice will be exposed to ETS using the system that has been developed under Dr. Davis's current contract. For exposure of neonatal mice, the system will be modified so that the animal ports are replaced with small chambers that will hold 2 adult and 12 suckling mice. Baffles will be added to the input to ensure mixing of smoke entering the chamber. Preliminary experiments will be done to verify the COHb levels in the suckling mice at each concentration of ETS to be used, and to determine whether any of the smoke characteristics, including particle size distribution, total particulate weight, CO concentration, and nicotine concentration, are altered by the modification or require longer to reach equilibrium. Dr. McCain of the Aerosol Sciences Division of Southern Research Institute will assist in design, installation, and evaluation of this modification.

(C) Analysis of data

Log transformed CFU data, arcsin transformed lesion indices derived from subjective scoring, and morphometric data will be analyzed by analysis of variance.

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Tukey's test will be used for post hoc comparisons among individual group means. Interactions as well as main effects will be evaluated. Reproducibility of smoke exposure will be assessed by determining coefficients of variation for carboxyhemoglobin results, total particulates, and nicotine content.

(D) Interpretation of results

For specific aim (i), effects of chronic ETS exposure on chronic low-dose *M. pulmonis* disease, significant increases in lesion severity would strongly suggest that the early increases in mycoplasmal numbers in the lungs of ETS exposed mice are linked with increased susceptibility to chronic lung disease. By comparison of results with those of studies now in progress, we will be able to assess the relative importance in adult mice of exposure before and after infection. Interpretation of results of experiments under specific aim (ii), effects of ETS exposure on pulmonary clearance of *M. pulmonis*, will be straightforward; inhibition of either intrapulmonary killing or physical clearance would provide a mechanistic explanation for the increased numbers of mycoplasmas in the lungs of ETS exposed adult mice. For specific aims (iii) and (iv), effects of prenatal and postnatal exposure to ETS, increases in either lesion severity or numbers of organisms in respiratory organs will indicate increased susceptibility in ETS exposed mice. Including a group exposed to CO in the experiments conducted under specific aim (iii) also will allow us to determine whether any effects of prenatal ETS exposure could be due to hypoxia. Results of experiments under specific aim (v) will be interpreted in conjunction with those of specific aims (iii) and (iv) to determine whether combined prenatal and postnatal exposure has greater effects on either Sendai virus or *M. pulmonis* disease than either prenatal or postnatal exposure alone.

(E) Timetable for the investigation

We expect that one year will be required to complete specific aims 1 (effects of chronic exposure on low dose mycoplasmal disease) and 2 (effects of chronic ETS exposure on pulmonary clearance). Specific aims 3 (effects of prenatal exposure to ETS) and 4 (effects of neonatal exposure to ETS) can be studied concurrently and will take approximately 1 year to complete. Specific aim 5 (synergistic effects of prenatal and neonatal exposure to ETS) will require 9 months to one year.

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10. AVAILABLE FACILITIES & RESOURCES

The proposed work will be conducted in the Department of Comparative Medicine at the University of Alabama at Birmingham. There is ample laboratory space for completion of this project, including 480 ft² culture and radioisotope laboratory space, 240 ft² histology and immunofluorescence laboratories, 240 ft² electrophoresis laboratory, 360 ft² medium preparation and centrifugation laboratory, 240 ft² microbiology laboratory,

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and 400 ft² cell culture laboratory. Drs. Schoeb, Davis, and Davidson each have 110 ft² of recently renovated office space in the Volker Hall building.

Available laboratory equipment includes a cytocentrifuge, sonicator, Leitz Orthoplan microscope with MTI video camera interfaced with an IBM compatible computer, autoclave, Barnstead Ultra-Pure reagent water system, Coulter counter, stomacher, motorized tissue homogenizer, Amicon concentrator, Leitz photomicroscope and fluorescence microscope, pH meters, microtomes, microtome knife sharpener, 2 Autotechnicon tissue processors, 5 BioGard hoods, 4 bacteriological incubators, CO_2 incubator, Leitz inverted microscope, 6 refrigerators, 3 -70°C freezers, Beckman tabletop refrigerated centrifuge, high speed centrifuge and ultracentrifuge, IEC refrigerated centrifuge, electrophoresis units, and cryostorage unit for cell culture.

The Department of Comparative Medicine employs an administrative assistant and a full-time secretary, who will be available for ordering supplies, accounts management, and printing of manuscripts.

All departments at UAB have access to an IBM 370-158 mainframe computer with Statistical Analysis System software. The Department of Comparative Medicine owns several IBM compatible desktop computers with word processing, spreadsheet, and statistical software.

Animal facilities include 1,000 ft² animal rooms of modern design that are available in a location convenient to our laboratories. The animal rooms have excellent environmental controls (100% fresh air with temperature regulation to ± 2°F). Charges for animal housing and ancillary necessities are prescribed by campus wide per diem rates which compare favorably with rates across the US. Additionally, Dr. Davis has a breeding colony of pathogen-free rats maintained in Trexler plastic film isolators from which breeding stock would be available for this project.

11. OTHER SUPPORT

Dr. Schoeb

Current:

1. NIH-DRR RR07003, "Laboratory Animal Medicine/Comparative Pathology" (Postdoctoral Training Program; J. R. Lindsey, PI); R (co-investigator); 07/01/88 to 06/30/92; R R This is a training grant; there is no overlap with this application.

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2. Veterans Administration, "Nonspecific Host Defenses in Respiratory Mycoplasmosis" (J. R. Lindsey, PI); R (co-investigator); 10/01/89 to 09/30/92; REDACTED R The general objective of this project is to investigate the role of host defenses that do not depend on specific immune recognition in the pathogenesis of murine respiratory mycoplasmosis. It does not duplicate this application in any way.

3. NIH-NIEHS N01-ES-05280, "Rodent Diagnostic Laboratory" (Contract, J. R. Lindsey, PI); R (co-investigator); 11-28-89 to 11-27-94; REDACTED / This is a contract to provide diagnostic evaluation of rodents for the NIEHS. It has no relationship to this application.

4. Center for Indoor Air Research, "Effects of Environmental Tobacco Smoke and NO_2 on Respiratory Infection: Murine Model Development" (J. K. Davis, Co-PI R (co-PI); 1/1/90 to 12/31/92; REDACTED This project is to develop a model in mice for study of effects of ETS on expression of murine mycoplasmosis and Sendai virus infection.

5. NIH-DRR RR00463, "Laboratory Animal Resource for Biomedical Research" (J. R. Lindsey, PI) R (co-investigator); 12-1-90 to 11-30-95; ADC REDACTED R The purpose of this project is to support pilot investigations of laboratory animal diseases that may be useful models of human diseases or are significant complications of animal experimentation. It in no way overlaps this application.

6. NIH-NIAID, "Animal Models of Human Viral Infections for Evaluation of Experimental Therapeutics" (Contract; E. R. Kern, PI); R (co-investigator); 1991-1996; R R This is a contract to evaluate potential new antiviral drugs. It has no relationship to this application.

7. NIH-DRR R24-RR05363-01, "CAR Bacillus Infection in Rats and Mice" (T. R. Schoeb, PI); R ; 7-1-91 to 6-30-96; REDACTED The purpose of this project is to determine whether or not cilia-associated respiratory (CAR) bacillus is a pathogen for rodents and to develop a method for detecting the infection if it is a pathogen. It in no way overlaps this application.

Pending:

NIH RO1-AI33164-01, "M. fermentans and the Progression of SIV Disease. Co-investigator, R 7-1-92 to 6-31-97, REDACTED The purpose of this project is to determine the role, if any, of *Mycoplasma fermentans*, in progression and severity of Simian immunodeficiency virus induced disease in macaques. It in no way overlaps this application. Approved, awaiting funding. J.K. Davis, PI.

EPA/EPSCoR, "Health Effects of Environmental Tobacco Smoke: Use of Biological

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Trenton R. Schoeb

Monitors for Risk Assessment," (J. K. Davis, Project Director); Investigator, REDACTED 10-1-92 to 9-31-97; REDACTED The proposed work includes an epidemiologic study using household pet dogs and cats as biological monitors to assess whether ETS exposure in the home is associated with increased risk of respiratory disease, and experimental studies of ETS exposure in rats to evaluate likely mechanisms by which ETS could exacerbate respiratory disease or provoke respiratory tract irritation. None of the work proposed duplicates any of the work described in this CIAR application.

Note: If this CIAR contract application is funded, the PI's commitment to one of the above projects will be adjusted so that his total commitment does not exceed 100%.

Dr. Davis

Current:

1. NIH, RR00463, "Laboratory Animal Resource for Biomedical Research;" REDACTED appointment (co-investigator); 12/1/90 to 11/30/95; REDACTED As described above; no overlap.

2. NIH, RR07003, "Laboratory Animal Medicine/Comparative Pathology;" REDACTED appointment (co-investigator); 07/01/88 to 06/30/92 REDACTED As described; no duplication.

3. Veterans Administration, "Nonspecific Host Defenses in Respiratory Mycoplasmosis" (unnumbered); R appointment (co-investigator); 10/01/89 to 09/30/92; REDACTED As described above; no relationship with this application.

4. Center for Indoor Air Research, "Effects of Environmental Tobacco Smoke and NO_2 on Respiratory Infection: Murine Model Development" (unnumbered); REDACTED (PI); 1/1/90 to 12/31/92; R As described above; no overlap.

5. NIH, ES-89-13, "Rodent Disease Diagnostic Laboratory;" R appointment (co-investigator); 10/1/89 to 9/30/94; R As described above; no duplication.

6. NIH, HL19741, "Murine CRD: A Model of Chronic Pulmonary Inflammation" R appointment (co-investigator); 7/1/88 to 6/30/92 R Purpose is to study traffic and functions of lymphocytes and macrophages in the lungs in mycoplasmosis; no relationship. REDACTED REDACTED

7. NIH-DRR R24-RR05363-01, "CAR Bacillus Infection in Rats and Mice", Co-investigator, R 7/1/91 to 6/30/96; REDACTED As described above; no overlap.

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Trenton R. Schoeb

Pending:

NIH RO1-AI33164-01, "M. fermentans and the Progression of SIV Disease. PI, REDACTED 7-1-92 to 6-31-97, REDACTED The purpose of this project is to determine the role, if any, of *Mycoplasma fermentans*, in progression and severity of Simian immunodeficiency virus induced disease in macaques. It in no way overlaps this application. Approved, awaiting funding.

EPA/EPSCoR, "Health Effects of Environmental Tobacco Smoke: Use of Biological Monitors for Risk Assessment," Project Director, R effort; 10-1-92 to 9-31-97, R REDACTED The proposed work includes an epidemiologic study using household pet dogs and cats as biological monitors to assess whether ETS exposure in the home is associated with increased risk of respiratory disease, and experimental studies of ETS exposure in rats to evaluate likely mechanisms by which ETS could exacerbate respiratory disease or provoke respiratory tract irritation. It does not overlap this application.

Dr. Davidson

Current:

1. NIH, RR00163, REDACTED oratory Animal Resource for Biomedical Research," 12/1/90 to 11/30/95; R Co-investigator, R . The purpose of this project is to support pilot investigations of laboratory animal diseases that may be useful models of human diseases or are significant complications of animal experimentation. J. R. Lindsey, PI.

2. NIH, RR07003, "Laboratory Animal Medicine/Comparative Pathology" (Postdoctoral Training Program); 07/01/88 to 06/30/92; REDACTED The purpose of this program is self-explanatory. J. R. Lindsey, PI.

3. Veterans Administration, "Nonspecific Host Defenses in Respiratory Mycoplasmosis" (unnumbered); 10/01/89 to 09/30/92; R Co-investigator, R The general objective of this project is to investigate the role of host defenses that do not depend on specific immune recognition in the pathogenesis of murine respiratory mycoplasmosis. J. R. Lindsey, PI.

4. NIH, ES8913, "Rodent Diagnostic Laboratory", 10/1/1989 to 09/30/94, REDACTED Investigator, R This is a contract to provide diagnostic evaluation of rodents for the NIEHS. J. R. Lindsey, PI.

5. NIH-DRR R24-RR05363-01, "CAR Bacillus Infection in Rats and Mice", Co-investigator,

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Trenton R. Schoeb

REDACTED 7-1-91 to 6-30-96; ADC $126,347; REDACTED. The purpose of this project is to determine whether or not cilia-associated respiratory (CAR) bacillus is a pathogen for rodents and to develop a method for detecting the infection if it is a pathogen.. It in no way overlaps this application. T. R. Schoeb, PI.

Pending:

NIH RO1-AI33164-01, "M. fermentans and the Progression of SIV Disease. Co-PI, REDACTED effort, 7-1-92 to 6-31-97; REDACTED The purpose of this project is to determine the role, if any, of *Mycoplasma fermentans*, in progression and severity of Simian immunodeficiency virus induced disease in macaques. It in no way overlaps this application. Approved, awaiting funding. J.K. Davis, PI.

EPA/EPSCoR, "Health Effects of Environmental Tobacco Smoke: Use of Biological Monitors for Risk Assessment," (J. K. Davis, Project Director); Investigator, R ; 10-1-92 to 9-31-97; REDACTED The proposed work includes an epidemiologic study using household pet dogs and cats as biological monitors to assess whether ETS exposure in the home is associated with increased risk of respiratory disease, and experimental studies of ETS exposure in rats to evaluate likely mechanisms by which ETS could exacerbate respiratory disease or provoke respiratory tract irritation. It does not overlap this application.

Dr. Dillon:

Current:

NIH/NIOSH, RO1 OHO2914-1, "On-Site Enumeration of Fungal Spores and Bacteria in Air," Principal Investigator R ; 2-1-92 to 1-31-95; REDACTED This project is to develop a rapid and accurate method for on-site assessment of microbial contamination of air in the workplace.

Pending:

EPA/EPSCoR, "Health Effects of Environmental Tobacco Smoke: Use of Biological Monitors for Risk Assessment," (J. K. Davis, Project Director), Co-investigator, R 10-1-92 to 9-31-97; REDACTED The proposed work includes an epidemiologic study using household pet dogs and cats as biological monitors to assess whether ETS exposure in the home is associated with increased risk of respiratory disease, and experimental studies of ETS exposure in rats to evaluate likely mechanisms by which ETS could exacerbate respiratory disease or provoke respiratory tract irritation. It does not overlap this application.

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EPA/EPSCoR, "Variability of Ozone and its Precursors in the Southeastern Environment," Co-investigator, R 1-1-93 to 12-31-93; R The purposes of this project are to develop a better understanding of the role of volatile organic compounds on generation of ozone and to evaluate variability in passive dosimetric techniques.

12. **BUDGET (contd.)**

(a) Salaries

T. R. Schoeb, DVM, PhD, ACVP. (PI; R) Dr. Schoeb will be responsible for the overall direction of the project, coordination of the experiments, and the reporting of the results of the studies. He will collate all data from the experiments and perform the statistical analyses of the data. He will be responsible for assessment of lesions and any additional evaluation, such as morphometric analysis, that may be necessary.

J. K. Davis, DVM, PhD, ACLAM (Co-investigator, R . Dr. Davis will oversee the animal colony, health assessment of the colony, and the exposures of mice to ETS and CO.

M. K. Davidson, MS, PhD, MT(ASCP) (Co-investigator, R **).** Dr. Davidson will oversee the exposures of mice to ETS and CO on a daily basis and will be responsible for the quality control methods involved in the exposures. She will assist Dr. Schoeb in daily collection and review of quality control data.

H. K. Dillon, MA, PhD (Co-investigator, R Dr. Dillon will oversee the sample collection and biochemical analyses of the simulated ETS. He will assist Dr. Davidson in the evaluation of the whole body exposure chambers for ETS and in the initial evaluation of the CO exposures.

K. C. Kervin, M.T.(ASCP). Research Assistant II. R Ms. Kervin has 10 years experience in research laboratories and 5 years experience in a microbiology laboratory. She has done many of the procedures outlined in this proposal and has some experience with the Kentucky Smoking Machine. Under the supervision of Drs. Schoeb and Davidson, she will be responsible for all analytical, microbiological, and quality control aspects of this project.

L. Thomas. Research Assistant I. R Ms. Thomas has 10 years experience as a research technician and has assisted Drs. Davis and Davidson establish ETS, aerosol and NO_2 exposure systems. Mice will need to be exposed daily to simulated ETS for nearly the entire project period. In addition, we will have to produce and care for experimental animals ourselves to avoid contamination with murine pathogens. Funds are requested to hire a research assistant I who will be responsible for daily exposure of the animals to ETS and animal care. A well trained individual is

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crucial in this position to avoid costly mistakes.

A yearly cost of living increase of 6% is requested for all personnel for years 2 and 3 according to UAB recommendations.

(b) Consultants

Dr. Joseph McCain of SRI has agreed to serve as a consultant to this project on an "as needed" basis. He provided invaluable assistance in developing the ETS expsure system for Dr. Davis's current contract, and has assisted Dr. Davidson in solving problems in aerosol sampling and data reduction for that project. For the proposed work, Dr. McCain will provide advice and assistance regarding technological aspects of smoke exposures to help assure consistent and well-characterized exposures, and his expertise will be necessary for installation of the whole-body chambers to expose infant mice and for any modifications to the Kentucky smoking machine that may be necessary for these experiments. We anticipate that his services will be needed about 10 hours in year 1, 40 hours in year 2 when the whole body chambers will be installed, and 10 hours in year 3.

(c) Supplies

Supplies were calculated by determining the total amount of supplies required for each experiment outlined in the proposal based on current prices and averaging them over the 3 years requested. Inflation for years 2 and 3 was estimated at 6% per year based on UAB recommendations. All items except animal costs are self explanatory.

Animal per diem charges are based on per diem rates of $0.51 to $0.59 per mouse cage per day, as projected by the university Animal Care Program for the three years of the project. These charges include a discount for investigators who provide all services within their animal rooms. We estimate that charges for about 1800 cage-weeks will be required to complete the project.

In addition to per diems for experimental animals, we must maintain sufficient breeder mice to produce the experimental animals. This was estimated at 20 cages (two isolators) of breeders that will have to be maintained. The cost per cage/day is about half of the above per diems because the cages are smaller. Production of animals is essential to ensure that the animals are not exposed to pathogens prior to experimental use and to maintain the pathogen-free status of the animals.

Isolator supplies are estimated at $1,000/year for each of the breeder isolators and include sealing tape, replacement gloves, fiberglass filter material, Alcide sterilant, replacement sleeves, spore strips for ensuring sterility of supplies, and water bottles. Maintenance estimates are based on our figures over the past 5 years.

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(g) Equipment

CO Analyzer ($5000, year 2). In year 2, mice will be exposed to CO sufficient to produce a carboxyhemoglobin concentration of 1% to 10% in exposed animals. We will be able to use Dr. Davis' whole body exposure chambers for the exposures, but do not have access to a CO analyzer that will be needed to monitor the exposures.

CO-Oximeter ($15,000, year 1). A CO-Oximeter is requested for measurement of carboxyhemoglobin (COHb) concentrations in mice. We have been using a spectrophotometer for these measurements, but it is very old and is becoming unreliable. After pricing suitable general purpose spectrophotometers ($28,000), we believe that purchase of a specialized instrument is the most cost effective method of obtaining this information. COHb measurements must be made several times a day on several mice as a measurement of the amount of simulated ETS, or CO, to which the mice are exposed. Additionally, the instrument must use a small sample size so that we may measure COHb concentrations in infant mice.

Electronic Aerosol Size Analyzer (EASA) ($27,000) and associated smoking equipment ($2000) (year 1). The EASA and associated small pieces of equipment currently in use in Dr. Davis's laboratory are owned by Southern Research and are being rented. Southern Research has committed this equipment to the laboratory until Dr. Davis's current project is completed, but they have indicated that they will need it back for an in-house project at that time. In modifying the Kentucky Smoking System for simulation of ETS, we have found the EASA critical for determining when the system has reached equilibrium prior to introducing animals and for minimizing day to day variability. The associated items are the necessary pumps, flow meters, gauges, mixing chambers, etc. that are needed for the EASA and for consistent delivery of the simulated ETS to the mice.

Isolators & associated equipment (years 2 and 3, $3000 each). We request funds each year to purchase 2 new, replacement isolators and associated fans, filter supports, doors, support boards, racks, replacement filter units, etc. for housing breeding animals. The expected life span of an Trexler plastic film isolator is about 1 year.

Nitrogen detector ($5000, year 1): Dr. Dillon of the Environmental Health Sciences has the necessary gas chromatography/mass spectrometry equipment for the quality control measurements required for this project, but does not have the required nitrogen detector needed for nicotine determinations. We request funds to purchase this instrument.

Smoke exposure chambers ($1000, year 2): We request funds to have the UAB machine shop construct the whole body exposure chambers needed to expose newborn mice to simulated ETS.

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13. BIOGRAPHICAL SKETCHES

Trenton R. Schoeb

Education:

Oklahoma State University	1972	BS (Pre-veterinary science)
Oklahoma State University	1974	DVM
Oklahoma State University	1977	MS (Veterinary pathology)
University of Alabama at Birmingham	1981	PhD (Experimental pathology)

Specialty certification: American College of Veterinary Pathologists

Scientific field: Experimental pathology; veterinary pathology

Major research interests: Pathogenesis of diseases of mucosal surfaces, particularly those of the respiratory tract; pathogenesis of respiratory diseases in general; diseases of laboratory rodents

Professional experience:

1. Associate Professor, Department of Comparative Medicine, University of Alabama at Birmingham, 1985-present.
2. Assistant Professor, Department of Comparative Medicine, UAB, 1981-1985.
3. Research Veterinarian (WOC), Birmingham Veterans Administration Medical Center, 1981-present.
4. Instructor, Department of Comparative Medicine, UAB, 1977-1981.

Publications:

1. Davidson MK, Lindsey JR, Brown MB, Schoeb TR, Cassell GH: Comparison of methods for detection of *Mycoplasma pulmonis* in experimentally and naturally infected rats. J Clin Microbiol 14:646-655, 1981.

2. Schoeb TR, Davidson MK, Lindsey JR: Intracage ammonia promotes growth of *Mycoplasma pulmonis* in rat respiratory tracts. Infect Immun 38:212-217, 1982.

3. Schoeb TR, Lindsey JR: Murine respiratory mycoplasmosis (MRM): Nasal passages, middle ears, larynx, trachea. *In*: International Life Sciences Institute Monographs on Pathology of Laboratory Animals: Respiratory System. Jones TC, Mohr U, Hunt RD (eds.); Springer-Verlag, Berlin, 1985; pp. 78-83.

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4. Schoeb TR, Lindsey JR: Murine respiratory mycoplasmosis (MRM): Lungs. *In*: International Life Sciences Institute Monographs on Pathology of Laboratory Animals: Respiratory System. Jones TC, Mohr U, Hunt RD (eds.); Springer-Verlag, Berlin, 1985; pp. 213-218.

5. Schoeb TR, Kervin KC, Lindsey JR: Exacerbation of murine respiratory mycoplasmosis in gnotobiotic F344/N rats by Sendai virus infection. Vet Pathol 22:272-282, 1985.

6. Lindsey JR, Davidson MK, Schoeb TR, Cassell GH: Murine mycoplasmal infections, diseases, and research complications–A critique of current knowledge. *In*: Complications of Viral and Mycoplasmal Infections in Rodents to Toxicology Research and Testing. Hamm TE Jr (ed.); Hemisphere Press Publishing Corp., Washington, DC, 1986: pp. 91-121.

7. Pinson DM, Schoeb TR, Lindsey JR, Davis JK: Evaluation by scoring and computerized morphometry of lesions of early *Mycoplasma pulmonis* infection and ammonia exposure in F344/N rats. Vet Pathol 23:550-555, 1986.

8. Schoeb TR, Lindsey JR: Exacerbation of murine respiratory mycoplasmosis by sialodacryoadenitis virus infection in gnotobiotic F344 rats. Vet Pathol 24:392-399, 1987.

9. Casebolt DB, Spalding DM, Schoeb TR, Lindsey JR: Suppression of immune response induction in Peyer's patch cells from mice infected with mouse hepatitis virus. Cell Immunol 109:97-103, 1987.

10. Pinson DM, Schoeb TR, Lin SL, Lindsey JR: Promotion of *Mycoplasma pulmonis* growth in rat tracheal organ cultures by ammonium chloride. Lab Anim Sci 38:143-147, 1988.

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12. Fallon MT, Schoeb TR, Benjamin WH Jr, Lindsey JR, Briles DE. Modulation of resistance to *Salmonella typhimurium* infection in mice by mouse hepatitis virus (MHV). Microbial Pathogenesis 6:81-91, 1989.

13. Schoeb TR, Fox JG. Enterocecocolitis associated with intraepithelial *Campylobacter*-like bacteria in rabbits (*Oryctolagus cuniculus*). Vet Pathol 27:73-80, 1990.

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14. Fallon MT, Benjamin WH Jr, Schoeb TR, Briles DE. Mouse hepatitis virus infection enhances resistance to *Salmonella typhimurium* in mice by inducing host suppression of bacterial growth. Infect Immun 59:852-856, 1991.

15. McDonald DM, Schoeb TR, Lindsey JR. *Mycoplasma pulmonis* infections cause long-lasting potentiation of neurogenic inflammation in the respiratory tract of the rat. J Clin Invest 87: 787-799, 1991.

16. Davis JK, Davidson M, Schoeb TR. Murine Respiratory Mycoplasmosis: A Model to Study Effects of Oxidants. Health Effects Institute Research Report No. 47, Health Effects Institute, Cambridge, MA, 1991.

17. Davis JK, Davidson MK, Schoeb TR, Lindsey JR. Decreased intrapulmonary killing of *Mycoplasma pulmonis* after short-term exposure to NO_2 is associated with damaged alveolar macrophages. Am Rev Resp Dis 145:406-411, 1992

18. Nichols PW, Schoeb TR, Davis JK, Davidson MK, Lindsey JR. Pulmonary clearance of *Mycoplasma pulmonis* in rats with respiratory viral infections and in rats of susceptible genotype. Lab Anim Sci, in press

19. Schoeb TR, Juliana MM, Nichols PW, Davis JK, Lindsey JR. Effects of viral and mycoplasmal infections, ammonia exposure, vitamin A deficiency, and age on adherence of *Mycoplasma pulmonis* in cultured rat tracheas. Submitted.

20. Liang S, Davis JK, Schoeb TR, Simecka JW, Cassell GH, Lindsey JR. Differential susceptibility of LEW and F344 rats to respiratory tract lesions of Sendai and sialodacryoadenitis virus infections. Submitted.

Jerry K. Davis, DVM, PhD

Education:

University of Tennessee	BS	1970	Animal Husbandry
Auburn University	DVM	1974	
Univ. of Alabama at Birmingham	PhD	1981	Exptl. Pathology

Specialty Certification: American College of Laboratory Animal Medicine

Scientific Field: Experimental pathology, immunology, respiratory tract diseases

Research Interests: Respiratory tract diseases and immunology; effects of air pollutants

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Appointments:

1974-1981	Doctoral Fellow, Department of Comparative Medicine, University of Alabama at Birmingham, Birmingham, AL.
1981-1985	Research Assistant Professor, Department of Comparative Medicine, University of Alabama at Birmingham, Birmingham, AL.
1985-present	Associate Professor, Department of Comparative Medicine, University of Alabama at Birmingham, Birmingham, AL.
1985-present	Research Assistant Professor, Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL. (II° appointment)
1990-present	Professor, Department of Comparative Medicine, University of Alabama at Birmingham, Birmingham, AL.

Publications

1. Davis, J.K., Delozier, K.M., Asa, D.K., Minion, F.C., and Cassell, G.H.: Interactions between murine alveolar macrophages and *Mycoplasma pulmonis in vitro*. Infect. Immun. 29:590-599, 1980.

2. Davis, J.K. and Cassell, G.H.: Murine respiratory mycoplasmosis in LEW and F344 rats: strain differences in lesion severity. Vet. Path. 19:280-293, 1982.

3. Davis, J.K., Parker, R.F., White, H., Dziedzic, D., Taylor, G., Davidson, M.K., Cox, N.R., and Cassell, G.H.: Strain differences in susceptibility to murine respiratory mycoplasmosis in C57BL/6 and C3H/HeN mice. Infect. Immun. 50:647-654, 1985.

4. Davis, J.K., Simecka, J.W., Williamson, J.S.P., Ross, S.E., Juliana, M.M., Thorp, R.B., and Cassell, G.H.: Nonspecific lymphocyte responses in F344 and LEW rats: susceptibility to murine respiratory mycoplasmosis and examination of cellular basis for strain differences. Infect. Immun. 49:152-158, 1985.

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6. Simecka, J.W., Davis, J.K., and Cassell, G.H.: Distribution of Ia antigens and T lymphocyte Subpopulations in rat lungs. Immunol. 57:93-98, 1986.

7. Williamson, J.S.P., Davis, J.K., and Cassell, G.H.: Polyclonal activation of rat splenic lymphocytes after *in vivo* administration of *Mycoplasma pulmonis* and its relation to *in vitro* response. Infect. Immun. 52:594-599, 1986.

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15. Ross, S.E., Davis, J.K., and Cassell, G.H.: Induction of hyper Ia expression on F344 rat B lymphocytes by *Mycoplasma pulmonis* mitogen. Zbl. Bakt. Suppl. 20. Recent Advances in Mycoplasmology, 584-592. 1990.

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19. Schoeb TR, Juliana MM, Nichols PW, Davis JK, Lindsey JR. Effects of viral and mycoplasmal infections, ammonia exposure, vitamin A deficiency, and age on adherence of *Mycoplasma pulmonis* in cultured rat tracheas. Submitted.

20. Liang S, Davis JK, Schoeb TR, Simecka JW, Cassell GH, Lindsey JR. Differential susceptibility of LEW and F344 rats to respiratory tract lesions of Sendai and sialodacryoadenitis virus infections. Submitted.

Maureen K. Davidson

Education:

University of Alabama at Birmingham	BS	1972	Biology
University of Alabama at Birmingham	BS	1973	Med. Tech.
University of Alabama at Birmingham	MS	1981	Exptl. Pathol.
University of Alabama at Birmingham	PhD	1988	Exptl. Pathol.
University Hospital, University of Alabama Hospitals			Med. Tech. Cert. 1973

Scientific Field: Experimental pathology, Infectious Diseases, Microbiology, Mycoplasmology, Immunology, Respiratory Tract Diseases

Major research interests: Host-parasite relationships in infectious diseases, host defence mechanisms, mycoplasmal diseases, virulence mechanisms, pathogenesis of disease, respiratory diseases.

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Professional experience:

1970-1972	Research Technician, Department of Anatomy, Dr. Edwin M. Weller, Ph.D., University of Alabama at Birmingham Medical Center.
1970-1972	Laboratory Instructor, Department of Biology, University of Alabama at Birmingham, University College.
1972	Laboratory Instructor in Genetics Laboratory, Department of Biology, University College, University of Alabama at Birmingham.
1972-1977	Clinical Instructor for Medical Laboratory Technician and Medical Technologist students, Microbiology Section, University Hospital, University of Alabama Hospitals, SPAH Programs.
1973-1974	Staff Technologist, Stat Laboratory, University Hospital, University of Alabama Hospitals, 1973-1974.
1974-1976	Staff Technologist, Clinical Laboratories, Microbiology Section, University Hospital, University of Alabama Hospitals.
1974-1977	Clinical Instructor for Clinical Pathology residents in Microbiology Section, University Hospital.
1974-1977	Clinical Instructor for medical and dental students, Microbiology Section, University Hospital.
1976-1977	Technical Supervisor, Clinical Laboratory, Microbiology Section, University Hospital, University of Alabama Hospitals.
1977-1981	Research Assistant, Microbiology/Clinical Pathology, Department of Comparative Medicine, University of Alabama at Birmingham.
1978-	Microbiologist, Veterans Administration Medical Center, Birmingham, AL.
1981-1987	Instructor, Department of Comparative Medicine, University of Alabama at Birmingham.
1987-	Assistant Professor, Department of Comparative Medicine, University of Alabama at Birmingham.
1988-	Director, Clinical Veterinary Laboratory, University of Alabama at Birmingham.

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Publications:

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10. Davidson, M.K., Lindsey, J.R., and Davis, J.K.: Requirements and selection of an animal model. Isr. J. Med. Sci. 23:551-555, 1987.

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11. Watson, H.L., Davidson, M.K., Cox, N.R., Davis, J.K. and Cassell, G.H.: Protein variability among strains of *Mycoplasma pulmonis*. Infect. Immun. 55(11):2838-2840, 1987.

12. Davidson, M.K., Lindsey, J.R., Parker, R.F., Tully, J.G., and Cassell, G.H. Differences in virulence among strains of *Mycoplasma pulmonis* for mice. Infect. Immun. 56(8):2156-2162, 1988.

13. Cox, N.R., Davidson, M.K., Davis, J.K. and Cassell, G.H.: Natural mycoplasmal infections in isolator-maintained LEW/Tru rats. Lab. Ani. Sci. 38(4):381-388, 1988.

14. Davidson, M.K., Davis, J.K., Lindsey, J.R., and Cassell, G.H. Clearance of different strains of *Mycoplasma pulmonis* from the respiratory tract of C3H/HeN mice. Infect. Immun. 56(8):2163-2168, 1988.

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16. Davidson, M.K., Lindsey, J.R., Davis, J.K., Parker, R.F., Ross, S.E., Watson, H.L., Tully, J.G. and Cassell, G.H.: Alternative approach to identification of virulence mechanisms of *Mycoplasma pulmonis*. Zbl. Bakt. Suppl. 20. Recent Advances in Mycoplasmology, 695-697, 1990.

17. Davis, J.K., Parker, R.F., Davidson, M.K., Cassell, G.H., and Lindsey, J.R.: Pulmonary Clearance of *Mycoplasma pulmonis* in Mice. Zbl. Bakt. Suppl. 20. Recent Advances in Mycoplasmology, 819-822. 1990.

18. Davidson, M.K., Davis, J.K., Gambill, G.P., Cassell, G.H. and Lindsey, J.R.: Mycoplasmas of Laboratory Rodents. In: Diagnosis of Mycoplasmoses in Domestic Animals, H. W. Whitford, editor. (In Press).

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H. Kenneth Dillon

Education:

University of North Carolina	BS	1969	Chemistry
Vanderbilt University	MA	1973	Physical Chemistry
Vanderbilt University	PhD	1974	Physical Chemistry

Scientific Field: Air pollution, Analytical Chemistry, Chromatography, Environmental Chemistry

Major research interests: Air pollution, Environmental Health Effects

Professional experience:

1969-1970	Teaching Fellow, Vanderbilt University, Nashville, TN
1970-1973	Eastman Kodak Fellow, Vanderbilt University, Nashville, TN
1973-1974	Associate Chemist, Southern Research Institute, Birmingham, AL
1974-1978	Research Chemist, Southern Research Institute, Birmingham, AL
1978-1980	Senior Chemist and Program Manager for Analytical Methods Development, Southern Research Institute, Birmingham, AL
1980-1985	Head, Industrial Hygiene Chemistry Section, Southern Research Institute, Birmingham, AL
1981-1985	Associate Director, Industrial Hygiene Laboratory, Southern Research Institute, Birmingham, AL
1985-	Associate Professor of Environmental Health, University of Alabama at Birmingham.

Publications:

1. Boyd KW, Emory MB, Dillon HK. Development of personal sampling and analytical methods for organochlorine compounds. Choundhary, G., ed. Chemical hazards in the workplace: measurement and control. American Chemical Society (ACS Symposium Series No. 149): 49-64, Washington, DC, 1981.

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2. Dillon HK, Bryant ML: 1,2,4-Trichlorobenzene, 1,2,4,5-tetrachlorobenzene, and pentachlorobenzene, Method No. P&CAM 343. Taylor, D.G., ed. NIOSH manual of Analytical Methods, Vol. 7, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 82-100. Cincinnati, OH, August, 1981.

3. Dillon HK, Emory MB: p-Chlorophenol; Method No. P&CAM 337. Taylor, D.G., ed. NIOSH manual of Analytical Methods, Vol. 7. National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication NO. 82-100. Cincinnati, OH, August, 1981.

4. Dillon HK, James RH, Miller HC, Wendky AK: SATS POHC Sampling and Analysis Methods-Final Report. Prepared by Southern Research Institute, Birmingham, AL, and Battelle Columbus Laboratories, Columbus, OH, under Contract 68-02-2685 for Industrial Environmental Research Laboratory, USEPA, Research Triangle Park, NC, 1981.

5. Dillon HK, Emory MB: Laboratory Validation of Ozone Sampling with Spill-Proof Impingers. Final Report. Prepared by Southern Research Institute, Birmingham, AL for the American Welding Society, Miami, FL 1984.

6. Dillon HK, Fowler WK, Bryant ML, Martin JJ, Sarver EW: Sampling Technology: Final Report. Prepared by Southern Research Institute, Birmingham, AL for Chemical Research & Development Center, U.S. Army Armament, Munitions & Chemical Command, Aberdeen Proving Ground, Maryland. Publication No. CRDC-CR-84051, 100 pp, May 1984.

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8. Dillon HK: Specific adsorbents for chemical agent detectors and dosimeters; Final Report. Prepared by Southern Research Institute, Birmingham, AL, under Contract DAAK11-82-C-0109 for the U.S. Army Armament Research Development Command, Aberdeen Proving Ground (EAO), MD, 1985.

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11. Dillon HK, Rose VE, Davis KS: Chemical Agent and Simulant Dosimeter. Final Report to the US Air Force, Dec. 1986.

12. Ho M, Dillon HK: Biological Monitoring. Environ. Scii Technol. 20(2):124-127, 1986.

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14. Dillon HK, Ho MH: Biological monitoring of exposure top organophosphorus pesticides. In: Biological Monitoring of Exposure to Chemicals - Volume 1: Organic Compounds. Ho MH, and Dillon HK, eds., Chap 19, pp. 227-287. John Wiley & Sons, Inc., New York, 1987.

15. Rumph PF, Dillon HK: Reducing formaldehyde exposure in anatomy laboratories by regulating ambient temperature. Presented at the Am. Assoc. Vet. Anat., Orlando, FL, 1989.

16. Dillon HK, Rumph PF: Estimation of exposure to chemicals volatilized from solutions. Presented at the Am Ind Hygiene Conf, Orlando, FL, 1990.

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APPENDIX

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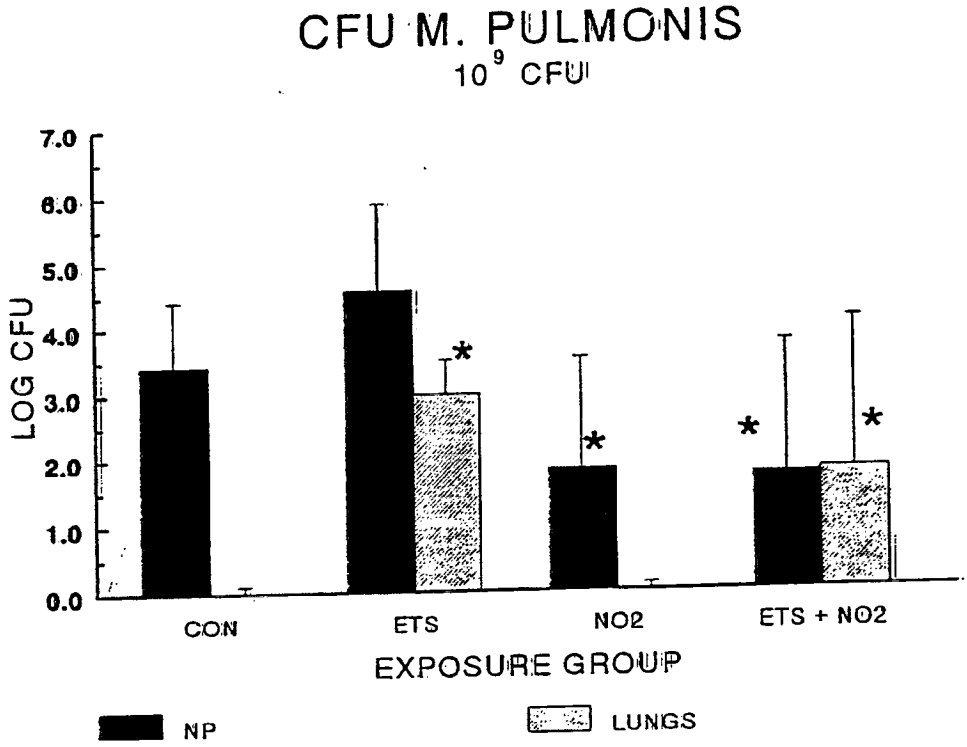

Figure 1 Numbers of *Mycoplasma pulmonis* (log colony-forming units) in lungs and nasal passages of mice inoculated with 0.10 50% pneumonia dose *M. pulmonis* and exposed to ETS, 2 ppm NO_2, or both (means ± standard deviations; n=6 for all groups). Means marked * are significantly different from controls ($P<0.001$)

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